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Morphological Variability and Nutritional Potential of *Zizyphus lotus* (L.) Lam: Assessment of Primary Metabolites and Mineral Composition Highlights Significant Valorization Prospects

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Abstract: In order to contribute to a better understanding of *Zizyphus lotus* nutritional composition in Morocco and to valorize this threatened species, morphological and chemical characterization of three fruit parts (pulp, seed, and kernel) of *Zizyphus lotus* from three different regions in Morocco was investigated. These ecotypes come from the Ain Chifa, Fez, and Guercif regions. Morphological characterization allowed the characterization of three populations of *Zizyphus lotus* fruit. Those from Fez and Ain Chifa have the largest fruit sizes. Thus, the largest pulp/fruit ratio is recorded in the populations of Ain Chifa and Guercif. Fruit color, which is the lightest, is important in the Ain Chifa population compared to the fruits of the two others. The chemical composition of the three fruit parts indicates that the pulp is richer in sugars, proteins, vitamin C, and minerals than the other two fruit parts. A comparison of the three ecotypes studied demonstrates that the chemical composition of the *Zizyphus lotus* fruit is influenced by climatic and geographical characteristics. We recorded that the population of Ain Chifa accumulates higher concentrations of proteins and vitamin C than those of Guercif and Fez. However, the Guercif population has high levels of total soluble sugars and minerals. The levels of soluble sugars, total soluble proteins, vitamin C, and minerals are not negligible, both in parts and populations, hence the need to preserve *Zizyphus lotus*, whose fruits can be exploited for the development of various foods and play an important role in human nutrition.

Keywords: *Zizyphus lotus*; morphology; chemical composition; primary metabolites; mineral elements.

1. Introduction

Due to its geographical location, Morocco benefits from favorable conditions for developing a rich and diverse flora, including a significant potential in Medicinal and Aromatic plants. There are more than 4200 plant species, of which 800 are endemic, among which only

280 species are used. Most are exported in the form of raw materials. Many medicinal and aromatic plants are widely used in various fields, including medicine, cosmetics, and other products [1].

Among the numerous medicinal plants, the genus Ziziphus from the Rhamnaceae family is often used in traditional medicine to treat and ease various health issues [2-4]. According to World Flora Online (WFO), this genus comprises 58 recognized plant species. *Zizyphus lotus* is a medicinal and aromatic plant extensively utilized in traditional medicine by local communities. [5]. This plant species has been traditionally used to treat and prevent various diseases, including diabetes, digestive issues, urinary tract problems, infectious diseases, cardiovascular conditions, neurological disorders, and skin ailments [6-14]. Numerous pharmacological studies have shown that extracts from different parts of *Z. lotus* exhibit significant bioactivities, including antioxidant, antimicrobial, hepato-nephroprotective, antihyperlipidemic, anti-inflammatory, analgesic, and antiproliferative effects. Additionally, several phytochemical studies have found that *Z. lotus* extracts are rich in natural compounds like polyphenols, flavonoids, terpenes, and phenolic acids. [15-19]. These phytochemical compounds are widely recognized in the scientific literature for their pharmacological effects against a variety of diseases. In this context, the shrub *Z. lotus*, commonly named 'Sedra', is the subject of numerous ongoing laboratory studies; this plant is part of the Rhamnaceae family, which comprises approximately 550 species across 45 genera. *Z. lotus* [20,21], a plant found in tropical and subtropical climates, is typically found in arid and semi-arid areas [22].

Z. lotus is a versatile shrub particularly valuable to those living in arid regions. It has traditionally been employed to treat various ailments, including diarrhea, and help regulate blood sugar levels[23]. Additionally, its tonic febrifuge fruit is utilized to dissolve kidney stones [24].

It seems that *Z. lotus* plays an important role in fixing mobile substrates by emitting branches outside the soil, in addition to the role it plays in preserving arid ecosystems. The plant of this species can reach significant dimensions in the absence of any aggression [25]. It also provides shelter for many animals (rodents, insects, and reptiles) and allows the establishment of a nitrophilous flora [26]. Despite its many advantages, *Z. lotus* is a neglected species in Morocco because there is little information available about this species, and it is often removed to make way for agricultural land.

The purpose of the study is to assess the nutritional value of different parts of *Zizyphus lotus* fruits from three different regions of Morocco. This will help to understand the relationship between the composition of the fruit and the geographical factors that affect the species.

2. Materials and Methods

2.1. Study sites.

The samples studied were from three populations of *Z. lotus* collected from different regions: Ain Chifa, Fez, and Guercif.

2.1.1. Guercif station.

Located in the region of the Oriental to the northeast of the Kingdom, it is characterized by an arid climate with low rainfall not exceeding 185 mm/year; its geographical location is Latitude: 34°13'32 "North, Longitude: 3°21'12" West, at an altitude of 367 meters. The region of Guercif benefits from fertile soil, which provides great agricultural wealth. This region seems to be the ideal environment for the proliferation of *Z. lotus*, given the floundering in areas with rainfall less than 500 mm.

2.1.2. Ain Chifa station.

It is situated near Imouzzer Kandar. Its geographical location is Latitude: 33°47'8 North, Longitude: 5°1'45 West, at 1084 meters above sea level. It is characterized by a continental-type climate, generally rainy winter and hot and dry summer. The average annual rainfall is about 460 mm, and the annual mean temperatures are 16°C with a maximum of 25°C and a minimum of 9,5°C.

2.1.3. Fez-Saïss station.

Located at the Saïss plateau between the Middle Atlas and the pre-Rif, its geographical location is latitude: 34°02′13″ North, Longitude: 4°59′59 West to 403 m above sea level. The region of Fez-Saïss enjoys a continental climate, very hot and dry in summer and cold and damp in winter. The average precipitation is about 375 mm/year, and the conditions for that station are favorable for developing agricultural activities. The temperature ranges between 4°C and 43°C.

2.2. Plant material.

The study was conducted on fruits of *Z. lotus* from three populations located in different bioclimates in Morocco. The populations come from the regions of Aïn Chifae, Fez, and Guercif. The different parts of the fruits that were studied were pulp, seed, and kernel. These parts were separated using a hammer and then ground using a mortar to increase the surface area of exchange between the solid and the solvent. This helps to facilitate extraction, which is the process of removing the desired compounds from the plant material.

2.3. Morphological characterization.

This part of the study characterized the morphological traits of *Z. lotus* fruits. It was conducted by collecting samples of 100 fruits from several populations of *Z. lotus*. The researchers measured the following traits for each fruit:

Caliber: The diameter of the fruit, measured in millimeters;

Weight: The weight of the fruit, expressed as a percentage of the total weight of the fruit;

Shape: The shape of the fruit, either globular (round) or oval;

Odor: The intensity of the odor of the fruit, either weak or strong;

Color: The color of the fruit, either light brown or dark brown.

2.4. Biochemical characteristics measured.

2.4.1. Total soluble sugars.

The assessment of the number of oses present in polysaccharides is based on the measurement of total soluble sugars using the method of Bachelier *et al*. [27]. This method consists of grinding 100 mg of fresh material with 4 ml of 80% ethanol. The content is incubated in a water bath, stirring for 30 minutes at 80°C, and the extract is centrifuged at 4500 rpm for 10 minutes. Total soluble sugars are then measured using anthrone. Absorbance was determined using a spectrophotometer at 625 nm (CHROM TECH V1200).

2.4.2. Soluble total protein.

Lowry method involves mixing 200 mg of fresh, ground plant material in 5 mL of sodium phosphate buffer (100 mM, pH 7.5), followed by centrifugation at 4000 rpm at 4 °C for 10 minutes. 0.2 mL of supernatant is recovered and added to the Lowry reagent, then left for 10 minutes at room temperature. Then, Folin is added to the mixture [28]. The reading is performed by spectrophotometry (CHROM TECH V1200) at 750 nm.

2.4.3. Vitamin C assay.

Determination of vitamin C content is carried out by iodometric titration, following the European standard (NF ISO 3961 9-96), which is based on redox reactions; ascorbic acid is added to an excess of iodine. A reaction occurs between the iodine and ascorbic acid:

$$\mathbf{C6H8O6\ (aq) + I2\ (aq) = C6H6O6\ (aq) + 2\ H+\ (aq) + 2\ I-(aq)} \qquad (1)$$

The excess iodine will then react with the starch present in the solution, giving it a dark blue color. The concentration of vitamin C in the samples is determined by a maceration followed by a titration. The maceration consists of mixing 1 g of powdered fresh material with 100 ml of distilled water and leaving it for 1 hour at 100°C under agitation. After adjusting the volume to 100 ml, 1 ml of a colored indicator (starch) is added to 10 ml of the solution. The titration of the samples was carried out using an iodine solution, which is added until a dark blue color appears.

2.5. Identification of mineral elements.

The method used to identify mineral elements is ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry), which is a technique that uses plasma to excite the elements in a sample [29]. The resulting emission lines are then measured to determine the amount of each element present. To analyze the sample, it is first dissolved in a mixture of nitric and perchloric acids. The solution is then nebulized and introduced into a plasma, where the temperature is high enough to vaporize the elements. The excited elements then emit light at wavelengths that are characteristic of each element. A spectrometer measures the intensity of the emitted light at each wavelength. The intensity is proportional to the amount of the element present in the sample. This allows for the quantitative and qualitative analysis of the elements. Mineral elements are determined by ICP-AES after mineralization of 100 mg of dry matter in 10 ml of a mixture containing nitric acid and perchloric acid for 4 hours at 120°C.

2.6. Statistical analysis.

The data obtained were subjected to a statistical analysis to investigate the existing variability between the different parts of fruits used and between the three populations (Ain Chifa, Fez, and Guercif). The data were processed using the "SYS-TAT 12" software. A mean comparison test was performed each time there was a significant effect of the factor studied by ANOVA. Fisher's F test is used to verify the hypothesis of equality of means at the 5% risk level.3.

3. Results and Discussion

3.1. Morphological study.

The parameters studied on the fruits of *Z. lotus* are reported in Table 1. The fruit size varies depending on the harvest site. It is about 11.70, 11.86, and 11 mm, respectively, for the three populations of Ain Chifa, Fez and Guercif. In addition, we noticed that the pulp is the most abundant part of the *Z. lotus* fruit in all three populations studied. It represents 59%, 55%, and 60% of the total fruit weight, respectively, for the populations of Ain Chifa, Fez, and Guercif. On the other hand, the seeds of *Z. lotus* vary from 6% to 7% of the total fruit weight in the three populations studied.

Table 1. Morphological analysis of the fruit of the three studied populations.

Characters	**Ain Chifa**	**Fez**	**Guercif**
Caliber (mm)	11.71 ± 1.04	11.86 ± 0.09	11 ± 1.15
Pulp-to-Fruit ratio (%)	58.72 ± 0.56	54.89 ± 0.78	59.82 ± 1.74
Seed/Fruit ratio (%)	7.33 ± 0.28	7.25 ± 0.40	6.29 ± 0.143
Kernel/Fruit ratio (%)	33.95 ± 0.80	37.86 ± 0.43	33.89 ± 0.143
Fruit color (%)	Claire: 75 Dark: 25	Claire: 64 Dark: 36	Claire: 58 Dark: 42
Shape	Oval	Oval	Oval
Odor	Strong	Strong	Strong

The fruits are brown to dark brown. At the Ain Chifa station, they are lighter in color (75%) than at the Fez (64%) and Guercif (58%) stations. The fruits are generally oval in shape and have a strong odor for all three populations of *Z. lotus*.

This analysis shows no significant difference between the three populations, either in terms of size (ddl = 2; F = 0.53; p = 0.589) or ratios (pulp, seed, and kernel weight relative to total fruit weight) (ddl = 2; F = 0; p = 1).

3.2. Biochemical characteristics.

3.2.1. Total soluble sugars content (TSS).

The results obtained are shown in Figure 1. They highlighted the variation in the TSS content in the three parts of the fruit in the three populations studied (Ain Chifa, Fez, and Guercif). We noticed that the pulp is the richest part of TSS, with contents ranging from 64.81 to 76.02 mg/g. Furthermore, the Guercif population had the highest content, while the Ain Chifa population had the lowest content. In terms of TSS content, the kernel is in second place, with contents ranging from 6.32 to 16.71 mg/g.

The guercif population is the richest in TSS compared to the other two populations. Indeed, the TSS contents in the seed are 8.92, 9.01, and 6.84 mg/g, respectively, for the Ain Chifa, Fez, and Guercif populations.

The variance analysis for TSS content shows a significant effect (F = 4.2; ddl = 2; $P \leq 0.05$) between the three *Z. lotus* populations and a highly significant effect at the level of the different parts of *Z. lotus* (F= 635.0; ddl =3; $P \leq 0.001$).

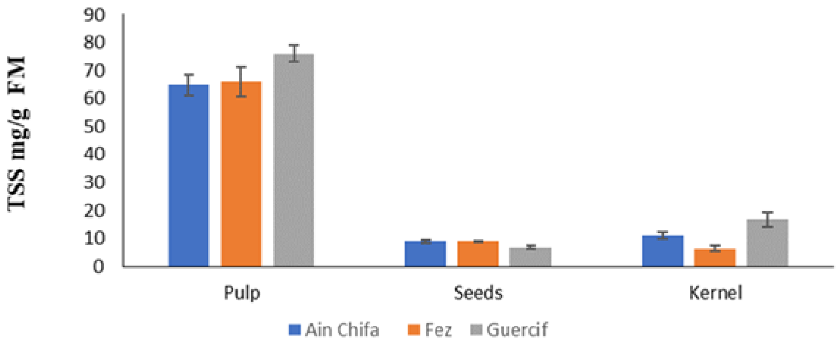

Figure 1. Variation in the TSS content in three populations of *Z. lotus* for n=3.

3.2.2. Total soluble protein content.

The results of this study show that the pulp of *Z. lotus* is the richest part in total soluble proteins, followed by the seed and, finally, the kernel. The Ain Chifa population accumulates high concentrations of total soluble proteins compared to the populations of Fez and Guercif for the tree-studied parts (Figure 2). In the pulp, we recorded total soluble protein contents of 4.1, 1.9, and 1.6 mg/g, respectively, for the Ain Chifa, Fez, and Guercif populations. In addition, the seed had values of 1.4, 1.2, and 1.4 mg/g, respectively, for the Ain Chifa, Fez, and Guercif populations. Meanwhile, for the kernel, the Ain Chifa population had the highest value (1.7 mg/g) compared to the two other populations.

The analysis of variance for total soluble protein content revealed a highly significant effect ($P < 0.001$), both for the population factor ($F = 74.6$; ddl = 2) and the fruit parts factor ($F = 109.485$; ddl = 2).

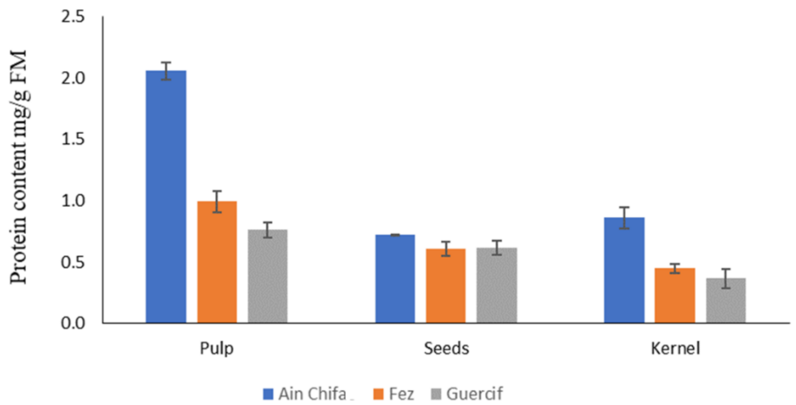

Figure 2. Variation in the content of soluble proteins in three populations of *Z. lotus* for n=3.

3.2.3. Vitamin C conten.

The dosage of vitamin C in the fruit of *Z. lotus* revealed a remarkable difference between the tree populations and between the parts of the fruit (Figure 3). The highest vitamin C content is recorded in the pulp. The values were of the order of 0.68, 0.38, and 0.04 mg/g, respectively, for the Ain Chifa, Fez, and Guercif populations. Regarding the seed, the vitamin C levels reached 0.21 mg/g for the Ain Chifa and Guercif populations, while the Fez population had a vitamin C content of 0.24 mg/g. The kernel had the lowest vitamin C content, at 0.12 mg/g, in the Ain Chifa and Guercif populations, while the Fez population has only 0.08 mg/g.

The statistical analysis of the results showed highly significant differences both between the populations ($F = 12$; ddl = 2; $P < 0.001$) and between the parts of the fruit studied ($F = 183$; ddl = 2; $P \leq 0.001$).

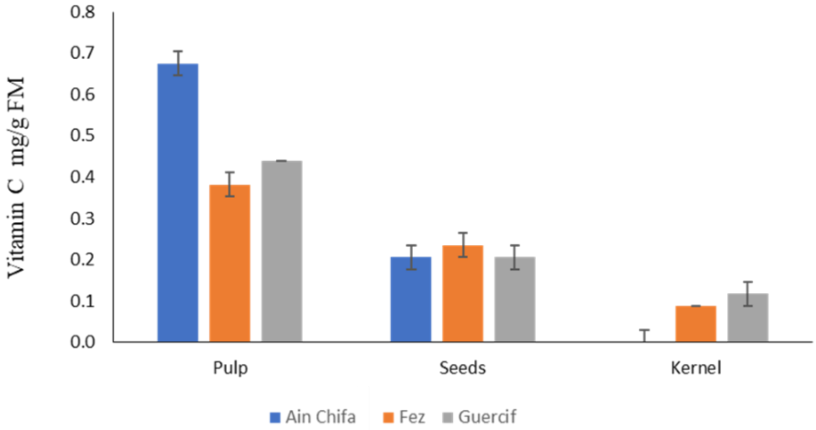

Figure 3. Variation in vitamin C content in three populations of *Z. lotus* with n=3.

3.3. Mineral elements.

Analysis of Table 2 showed a large variation between the three parts of the fruit and the three populations studied. Calcium, potassium, phosphorus, magnesium, and sodium are the major mineral elements in *Z. lotus* fruits. In fact, zinc and copper are the lowest elements.

Calcium has values between 157.830 and 486.815 mg/100g in the pulp, with the Fez population having the highest level (486.815 mg/100g). The seed contains between 145.572 and 396.203 mg/100g, while the kernel contains between 132.012 and 416.460 mg/100g. The Guercif population is the richest in this element, with respective levels of 396.203 and 416 mg/100g in the seed and kernel.

Regarding potassium, it has variable levels between 264.305 and 655.356 mg/100g in the pulp, of which the Fez population is the richest in this element, with a level of 655.356 mg/100g. However, the seed has levels between 109.588 and 529.731 mg/100g, and the kernel has values between 111.253 and 297.253 mg/100g. The Guercif population is the richest, with levels of 529.731 mg/100g and 297.869 mg/100g, respectively, for the seed and kernel.

The phosphorus content varies from 47.341 to 60.315 mg/100g in the pulp, from 8.475 to 456.368 mg/100g in the seed, and from 14.381 to 26.535 mg/100g in the kernel, of which the highest level is recorded in the Guercif population for the three parts of the fruit of *Z. lotus*.

The magnesium content is between 34.584 and 148.723 mg/100g, 16.471 and 285.102 mg/100g, and between 25.787 and 140.417 mg/100g, respectively, in the pulp, seed, and kernel of *Z. lotus*. Fez population is the richest in this element in the pulp (148.723 mg/100g). At the seed level, the highest level is that of Ain Chifa (285.102 mg/100g). At the kernel level, the Guercif population is at a higher level than the two other populations (140.417 mg/100g).

Sodium is present, with levels ranging from 26.159 to 73.696 mg/100g, 30.019 to 90.250 mg/100g, and between 33.545 and 94.754 mg/100g, respectively, in the pulp, seed, and kernel. The highest sodium content in the pulp and seed is found in the Guercif population. However, the seed of the Ain Chifa population is the richest compared to the other two populations.

Zinc is recorded with levels of the order of 0 to 7.815 mg/100g, 0 to 13.042 mg/100g, and between 0 and 13.927 mg/100g, respectively, in the pulp, seed, and kernel of *Z. lotus*. The Guercif population is the richest in this element in all three fruit parts, with the absence of this element in the pulp of Ain Chifa and in the seed and kernel of Fez.

We recorded copper levels of between 5.789 and 13.031 mg/100g, 3.412 mg/100g, and 17.32 and between 4.716 mg/100g, respectively, in the pulp, seed, and kernel of *Z. lotus*. The

Guercif population is rich in copper in all three parts studied. In comparison to the other two populations,

Based on the results obtained, we noticed that the Guercif population has predominantly high levels of mineral elements.

The statistical analysis of the results obtained shows highly significant differences, both between populations and between the fruit parts studied (df = 2; P ≤ 0.001) for all mineral elements.

Table 2. The mineral content of three populations.

Part (mg/100g)	Population	Ca	K	P	Mg	Na	Zn	Cu
Pulp	Ain Chifa	157.830	264.305	47.341	34.584	26.159	<0.010	5.918
	Fez	486.815	655.356	57.973	148.723	65.987	5.250	5.789
	Guercif	326.752	340.467	60.315	139.726	73.696	7.815	13.031
Seed	Ain Chifa	383.963	441.663	402.151	285.102	90.250	10.225	9.299
	Fez	145.572	109.588	8.475	16.471	30.019	<0.010	3.412
	Guercif	396.203	529.731	456.368	280.152	61.138	13.042	17.320
kernel	Ain Chifa	212.851	111.253	14.381	34.787	64.144	0.928	8.925
	Fez	132.012	207.555	19.405	25.641	33.545	< 0.010	4.716
	Guercif	416.460	297.869	26.535	140.417	94.754	13.927	28.593

The morphology of *Z. lotus* has been studied very little. The results obtained are contradictory to those found by Abdeddaim *et al*. [30], who recorded 50%, 42.7%, and 7.29%, respectively, for the pulp/fruit, kernel/fruit, and seed/fruit ratios. The caliber was 11.9 mm. Boudraa *et al*. [31] obtained a value of 55.36% for the pulp/fruit ratio, while the caliber was 14.65 mm.

Regarding total soluble sugars, results in our study are low compared to those found in the pulp and seed of Algerian *Z. lotus* studied by Abdeddaim *et al*. [30], who obtained a TSS content of 105.5 mg/g for the pulp and 41.0 mg/g for the seed. As for *Z.lotus* pulp, the content obtained is lower than that found for the species *Ziziphus jujuba* studied by Li *et al*. [32], which recorded a content of 80.6 mg/g. Total soluble sugars are an important source of energy for the body. They can also help to regulate blood sugar levels and promote digestive health.

On the topic of proteins, and based on our results, we found that the pulp is the richest part of total proteins compared to the seed part. These results are in contradiction with those obtained by Abdeddaim *et al*. [31], who found a total soluble protein content of 11.8 mg/g in the pulp, while it is 142.2 mg/g in the seed of the same species but of Algerian origin. In fact, Chouaibi *et al* [33]. Recorded a content of 191.1 mg/g for the seed part. Recently, Khair *et al*. [34] worked on the seed of *Zizyphus spina*-christi, and they found a value of 160.3 mg/g in total soluble protein. However, the results obtained are higher than those of Laamouri *et al*. [35]. In fact, by analyzing the fruits of three species of Zizyphus (*Z. lotus*, Z. jujuba, and Z. spina christi), the latter noted proportions of 1.9 mg/g, 4.6 mg/g, and 8.8 mg/g, respectively. Berry-Koch *et al*. [36] found 48.0 mg/g in the pulp of Z. spina-christi.

Vitamin C levels in our study are very high compared to those obtained by Boudraa *et al*. [31], who worked on the same species. He found 0.06 mg/g of vitamin C content in the pulp. Similarly, the study by Li *et al*. [32] recorded a vitamin C content that varies between 0.36 mg/g and 0.19 mg/g. Chouaibi *et al*. [33] worked on the seed of the same species but of Algerian origin. They found it is rich in vitamin C with a content of 0.31 mg/g at the seed level.

Concerning mineral composition, the pulp of *Z. lotus* is rich in mineral elements, essentially potassium, calcium, magnesium, phosphorus, and sodium. This idea was confirmed by Li and his collaborators [32]. Boudraa and his collaborators [31] proved that this organ contains 134 mg/100g of potassium, 490.84 mg/100g of calcium, and 397.91 mg/100g of magnesium. In 2014, Abdeddaim and his collaborators [30] found that the mineral fraction at the level of the pulp is composed of magnesium, calcium, phosphorus, sodium, manganese, iron, and zinc, with respective contents of 397.91, 134.99, 10.62, 11.45, 2.17, 1.33, and 0.44 mg/100g.

Regarding the seed, Chouaibi and his collaborators [33] recorded the presence of magnesium, with a content of 397.91 mg/100g, of calcium, whose content is 490.84, 134.99, 1.33, 2.17, and 0.44 g/100g, respectively for potassium, iron, manganese, and zinc. Abdeddaim *et al* [30] detected, at the level of the seed of *Z. lotus*, the presence of magnesium with a very high content of 1349 mg/100g followed by calcium, whose content is 97.92 mg/100g, phosphorus (10.62 mg/100g), sodium (17.41 mg/100g), manganese (7.84 mg/100g), iron, and zinc, with respective contents of 1.21 mg/100g and 1.38 mg/100g. Other previous studies conducted by Li and his collaborators [32] found a composition (expressed in mg/100g) formed by potassium (458), calcium (118), manganese (51.2), sodium (7.61), zinc (0.63), and iron (0.42).

4. Conclusions

In light of the results, we noted that the morphological parameters (size, weight of fruit different parts compared to the total weight of the fruit, and color of the fruit) vary depending on the fruit origin studied. Chemical characteristics are more likely to vary than morphological characteristics. This is because chemical characteristics are affected by a wider range of environmental factors, such as climate, temperature, and precipitation. *Z.lotus* fruit is a valuable source of nutrients. Further research is needed to fully understand the composition and usefulness of the different parts of the fruit and evaluate its long-term storage potential. Domestication of the species would also be beneficial for conducting large-scale analyses. Efforts should be made to plant more *Z. lotus* plants to increase the species' population. The pharmaceutical and cosmetic industries should be encouraged to use *Z. lotus* in their products, and cooperatives should be encouraged to use the plant to improve the local economy.

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Conflicts of Interest

The authors declare no conflicts of interest related to this article.

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